MATING TYPES IN DIVERSE RACES OF PARAMECIUM CAUDATUM

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Introduction

Investigations on *Paramecium aurelia* (Sonneborn, 1937, 1938 *a* and *b*), and on *P. bursaria* (Jennings, 1938 *a* and *b*, 1939 *a* and *b*) have recently shown that these species consist of a number of mating types. As a rule, and possibly always, individuals of the same mating type will not conjugate with each other; but when cultures of certain diverse mating types are mixed together there follows under appropriate conditions an immediate agglutinative reaction leading to conjugation between animals of diverse types. These phenomena are of interest in themselves, and in relation to sexuality and self-sterility; and they provide a means by which the genetics of these organisms may be rapidly developed. It therefore appears desirable to investigate from this point of view a large number of diverse species so that there may be available a broad comparative body of knowledge of these phenomena.

For this purpose, *Paramecium caudatum,* a species not hitherto studied from this point of view, was selected for intensive investigation. This species was chosen because it is one of the commonest and most intensively investigated species of Paramecium and because its nucleus and chromosomes are moderately favorable for the cytological work that must eventually become correlated with the genetic analysis.

Four major problems have been attacked experimentally. The first and basic problem is the occurrence, interrelation and geographical distribution of the mating types. The second problem, for which no final answer is available, is the inheritance of mating type during vegetative reproduction. The third is the influence of various environmental factors (nutrition, time of day, and temperature) on conjugation following mixture of different mating types. The final problem was to discover, if possible, morphological or physiological differences between the diverse groups that could be distinguished by their breeding behavior.

Materials

The material used in the present work was derived from collections of *Paramecium caudatum* obtained from twenty-six natural sources in

Canada and in the states of California, Connecticut, Georgia, Kansas, Maryland, Massachusetts, and Pennsylvania. Soon after each collection reached the laboratory, one or more individuals were isolated and from each individual a large stock culture was developed. These ninety-three stock cultures were the ones employed in all the following experimental work. All the clones used were identified as *P. caudatum* by examination of temporary aceto-carmine preparations or, in a few cases, of permanent Feulgen preparations to determine the number and type of micronuclei.

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Methods

The basic culture fluid, a lettuce infusion medium, was prepared as described by Sonneborn (1936), save that .75 grams of dried lettuce per liter was used instead of 1.5 grams. This fluid was lightly inoculated before use with a single unidentified species of bacteria grown on agar slants. This bacterium was isolated in the early stages of the work from a thriving culture of the paramecia.

The paramecia were cultured either as isolation lines on depression slides with daily transfer of single animals or as mass cultures in glass caster dishes with periodical transfer of a number of the animals to a fresh dish. In some cases, the mass cultures were fed by adding a grain of pearl barley, or a small piece of coagulated egg yolk to induce bacterial growth.

No effort was made to maintain absolutely sterile conditions but precautions were taken to insure the predominance of the desired bacterium in the culture. The glassware was sterilized by boiling or autoclaving, and the cultures were exposed to the air only long enough to allow the removal of animals for transfer or for experimental purposes.

At times, heavy bacterial growths (presumably of a contaminating bacterium) caused the appearance of heavy clouds of bacteria in the bottom of the slides or caster dishes. At other times, some of the cultures became contaminated by a small flagellate. The cultures were effectively purified of the contaminating organisms by running single animals in isolation lines for four days in succession. In making the transfers, the mirror of the microscope was tilted so that no light fell on the objectives and the contaminating organisms appeared as luminous dots. In

this way it was possible to draw back up into the pipette most of the contaminating organisms transferred with the paramecium. This method reduces considerably the risk of injury to the animals which the repeated transfers used in washing by the method of Parpart (1928) involve. Although the method described merely insures a predominance of the desired bacterium, it was found to be entirely satisfactory.

Testing Cultures for Mating Types

The fundamental observation on which the concept of mating types is based is simply this: certain cultures in which conjugation does not occur when separate, conjugate when mixed together. Two such cultures that do not conjugate alone but do conjugate when mixed are said to be of different mating types. In order to ascertain whether there occur in *P. caudatum* mating types such as those found in *P. aurelia, P. bursaria* and other species of Paramecium, it was necessary to obtain cultures within which conjugation did not occur, to mix representatives of these in all possible combinations of two, and to observe whether conjugation occurred in the mixtures or not.

The cultures to be tested for mating types were the 93 from the various collections mentioned in the section "Materials." Previous work by Sonneborn (1938*a*) and Jennings (1938*a*) on other species of Paramecium has shown that it is unnecessary to make all possible combinations of two among the cultures examined, for they found that all cultures of the same mating type behave alike when mixed with any other culture. Therefore, in the present work, after mating types had been discovered, only one representative culture of each mating type was used for mixture with new cultures of unknown mating type. The rule, therefore, was to mix every unknown culture with every other unknown culture and with representative cultures of each known mating type.

Occurrence, Number, and Interaction of the Mating Types

As a result of mixing the various clones it was found that certain mixtures regularly gave conjugation while others regularly gave no conjugation. It was concluded, therefore, that mating types were present in *P. caudatum*. This agrees with the findings of Giese and Arkoosh (1939) who reported the presence of two mating types in *P. caudatum*. When the results were collected, it was found that the clones studied could be divided into at least four and probably five groups of two mating types each. The groups were numbered one to five in order of their discovery. The mating types of Group 1 were designated I and II, those of Group 2, III and IV, those of Group 3, V and VI, those of Group 4, VII and VIII, and those of Group 5, IX and X. Although

this is the same nomenclature used by Sonneborn for *P. aurelia,* it implies no connection between the corresponding groups and types in the two species.

The interaction of groups and types in *P. caudatum* is shown in Table I. Each mating type in a group conjugates only with the other mating type in the group. Thus type I conjugates only with type II and not with other clones of type I or with clones of types III, IV, V, and VI, and so on for the other groups.

TABLE I

The relations among types and groups in *P. caudatum.* Conjugation is represented by a plus, absence of conjugation by a minus. A blank indicates that no mixture was made when both of the groups involved were known to be in reactive condition.

Group		1		2		3		4		5	
	Type	I	II	III	IV	V	VI	VII	VIII	IX	X
1	I	−	+	−	−	−	−				
	II	+	−	−	−	−	−				
2	III	−	−	−	+	−	−	−	−	−	−
	IV	−	−	+	−	−	−	−	−	−	−
3	V	−	−	−	−	−	+	−	−	−	−
	VI	−	−	−	−	+	−	−	−	−	−
4	VII			−	−	−	−	−	+	−	−
	VIII			−	−	−	−	+	−	−	−
5	IX			−	−	−	−	−	−	−	+
	X			−	−	−	−	−	−	+	−

It is not certain as yet that Group 1 is separate from both Group 4 and Group 5, since so far Group 1 has not been in condition to conjugate at the same time as Groups 4 and 5. However, from other considerations, it appears highly probable that there really are five groups of mating types. There remain, however, two collections from which no clones have so far conjugated when mixed with each other or with any of the groups of mating types. It is possible that these two collections may represent one mating type of a sixth group.

If samples of the two mating types in a group are mixed when in the proper physiological condition, there follows immediately the pronounced agglutinative mating reaction described by Sonneborn (1937) for *P. aurelia* and by Jennings (1939*a*) for *P. bursaria.* When the animals are put together they stick to those of the opposite mating type with which they chance to come in contact and form clumps which later break down into pairs which complete conjugation.

In regard to the geographical distribution of the various groups, it is to be noticed that Group 1 was found in two collections from Baltimore, Maryland, but not in collections from other localities. Group 2

was found in six collections from Baltimore; one collection from Woodstock, Md.; one collection from New Haven, Conn.; one collection from Stanford, California; and two collections from Falmouth, Mass. Group 3 was found in three collections from Baltimore, three from Baldwin City, Kansas, one collection from Atlanta, Georgia, and one collection from an unknown locality in Connecticut. Group 4 was found in one collection from Baltimore, one collection from Waterville, Conn., and one collection from New Haven, Conn. Group 5 was found in a collection from Hamden, Conn. The two collections whose group is still undetermined were from Philadelphia, Pa. and Canada near Buffalo, N. Y.

In general, it can be said that there is no definite evidence of geographically isolated non-interconjugating groups of mating types. It is true that Groups 1 and 5 have been found in only one general locality, but since there is little material available for these groups this cannot be considered significant. Animals of the other groups have been found in widely separate localities.

Conjugation Within a Clone

There are some apparent exceptions to the rule that any clone in a group conjugates with only one of the two mating types in the group. However, in all these apparent exceptions, conjugation has also occurred in one of the control cultures, so that it is not a question of a clone conjugating with clones of the two mating types of a group but rather a question of conjugation between members of the same clone. As an example, some results of mixtures of five clones of type IV (A2 to A6) with a type III clone (S1) and a type IV clone (A1) will be given. In the mixtures with the type III clone, from 61 to 72 pairs were formed with from 7 to 11 pairs of conjugants in the mixtures with the type IV clone. However, no conjugants were found in the type III (S1) control, while ten pairs were present in the type IV (A1) control. Some conjugants were found in the A2 to A6 controls, ranging from none in A6 to eleven in A4. Thus it is seen that the conjugation in the mixtures with A1 was the result of conjugation *within* the various clones and not the result of mixture.

In the clones in which conjugation has been observed to occur in the controls set up when making mixtures, conjugation has also been observed in the source cultures. Many of the cultures have conjugated at times without mixture. There is a great deal of variation with respect to this phenomenon among different clones. In some clones there have been large percentages of conjugants at intervals of two weeks to a

month. Each time the conjugation occurred in a culture which had been started from a single non-conjugant animal at the time of the previous occurrence of conjugation. The proportion of animals conjugating varied in these cultures from about 20 per cent to nearly 100 per cent. Other clones have conjugated at only one time during a period of nine to twelve months, and in these the proportion of animals conjugating varied from 5 per cent to 10 per cent. In still other clones, no conjugation has ever been observed except upon mixture with another clone of the proper mating type. Examples of the kind of clone in which large numbers of conjugants occur at short intervals have been found in both Groups 1 and 2. In Group 1, all three of the type II clones so far discovered are of this kind while in Group 2 two of the 7 type III clones and five of the 12 type IV clones are of this kind. Clones of the second kind in which small proportions of conjugants occur at long intervals have been found in two groups. In Group 1, there are four examples all belonging to type I, while in Group 3, two type V clones and two type VI cones are of this kind. In each of these, only a small proportion of the animals in one culture have conjugated at one time during the entire period of eight months to a year and a half that they have been under observation. Examples of clones which have never, while under observation, conjugated without mixture are found in all three groups: in Group 1, one type I clone; in Group 2, two type IV clones and one type III clone; and in Group 3, one type V and one type VI clone. The length of time that this condition has held true differs with different clones since some have been kept in the laboratory for longer periods than others. Four of the clones have been under observation for a year to a year and a half, and two for eight months.

Since conjugation does occur in this manner within a clone, the problem presents itself of whether or not two mating types have been produced within the clones as Sonneborn (1937) found in *P. aurelia* at autogamy. In order to test this matter, it was necessary to do two things: first to see if any autogamy or endomixis occurred (endomixis in *P. caudatum* has been reported by Erdmann and Woodruff, 1916, and by Chejfec, 1930), and if so, to see if conjugation in a culture without mixture depended upon its prior occurrence in the culture; second, to find out whether or not both of the mating types in a group were produced in a culture originally of one mating type and whether or not the differentiation into two types occurred at autogamy or endomixis.

In order to test the possibilities just mentioned, it was necessary to attack the problem in two ways. In the first place, to find if autogamy or endomixis were occurring, it was necessary to stain cultures daily with aceto-carmine. In pursuance of this plan, twenty-four clones were

run in daily isolation lines with daily staining for a period of thirty days. During this time one-fourth to one-half of the animals in each of the depressions were removed each day, stained with aceto-carmine and examined with the compound microscope for nuclear changes indicative of autogamy. Although examples of clones which had, in caster cultures, been producing conjugants at intervals of from ten days to two weeks were included, no evidence of any nuclear changes was observed during the month the cultures were under observation.

Although the results indicated that conjugation was occurring in these cultures without previous autogamy, it was felt, since the tests for autogamy were made under isolation line conditions in depression slides and the conjugation which had occurred had been under mass culture conditions, that the environmental conditions in the two situations were sufficiently different so that autogamy might have occurred in the parent caster dish cultures even though none occurred in the isolation lines derived from them. It was therefore decided to stain representative samples from a caster dish culture that was originally derived from one animal of clone D4, a clone in which conjugation occurred frequently. This animal was allowed to multiply in a depression slide. As soon as a sufficient number of animals was present, they were transferred to a caster dish and sixty drops of culture fluid added. As soon as several hundred animals were present in the dish, a grain of pearl barley was added to give a constant supply of food, since it was under these conditions that conjugation was previously observed to occur when the paramecia in the isolation lines showed no indication of autogamy.

The procedure used in testing for autogamy was to stain a sample from the culture every day and examine for nuclear changes. When only a relatively small number of individuals was present in the culture one-fourth were stained with aceto-carmine for the daily examination until at least ten were being stained. From that time onward from ten to one hundred animals were examined daily. No evidence of nuclear reorganization was ever observed in this culture but practically 100 per cent of the animals were conjugating twelve days after the start of the experiment.

These results indicate (for clone D4 at any rate) that the conjugation observed is not the result of a previously occurring autogamy. There remained to be answered, however, the question of whether or not both mating types were present in a clone when conjugation occurred. In order to answer this question, pairs of conjugants which were not yet firmly united were separated by squirting them violently from a small bore pipette. The cultures derived from the animals separated in this way are known as split-pair cultures. Because of the difficulty of finding

pairs which are not yet firmly united in cultures in which only a small proportion of the animals conjugated at one time during the course of the experiment, all the work with split pairs was carried on with those cultures in which large proportions of the animals conjugated at relatively short intervals. In Group 1, type II, clones D4 and C2 were used mainly with some additional work on clone D2. In clones D4 and C2, a number of pairs (17 in D4 and 18 in C2) were split and cultures grown from each member of the pair. Besides the split-pair cultures, a number of cultures were started from single non-conjugant animals from cultures in which conjugation was occurring. In Group 2, clone P (type III), twelve pairs of conjugants were split and cultures were grown from them. The clones were tested for mating type as soon as the population in the cultures had reached a sufficient density to make tests for type possible. However, conjugation again occurred in many of the cultures before it was possible to make the tests. The cultures which were tested were always of the same type as the parent culture. Among the split pairs from type II cultures, cultures from both members were tested and reacted in the case of two split pairs from D4 and one split pair from C2. Both members of each pair were type II, the same as the original culture. In the split-pair cultures from clone P both members of four pairs reacted as type III the same as the original culture. In addition, those split pairs in which only one of the resulting cultures gave a reaction and those cultures derived from isolated non-conjugating animals behaved as the same type as the parent cultures when tested for mating type.

In summary, it can be said that the results of the work on two clones in Group 1 (C2 and D4, type II) and one clone in Group 2 (P, type III) indicate that there is no permanent change of type when conjugation occurs within a culture since, whenever cultures from both members of a pair have given a test for mating type, both have been of the same type as the parent culture. Furthermore, the parent cultures still react as the same mating type they were when first isolated, even though they have been subcultured many times during the year to a year and a half that they have been in the laboratory.

These results do not allow any definite conclusion about possible temporary changes of mating type during vegetative reproduction such as Kimball (1939) found in *P. aurelia*. If such changes occur, they are temporary and the animals quickly revert to the mating type characteristic of the clone. The possibility that conjugation in these cases is between animals of the same mating type cannot be ruled out but seems unlikely in view of the previous work on Paramecium.

Although the results presented indicate that conjugation within a

clone (selfing) is not the result of a change of mating type following autogamy, there are several points of interest in connection with such selfings. They occur only in cultures which have been in the same culture dish for a period of time—two weeks approximately if a grain of pearl barley is added to the culture, longer if the cultures are fed by the addition of lettuce infusion. If animals from such selfing cultures are removed during the period before selfing occurs and mixed with the appropriate mating type conjugation will take place, however, and continues to occur even when selfing has commenced in the culture. It can thus be seen that being in the proper condition to conjugate when mixed is not enough to induce selfing and that some additional factor is involved. Successive selfings can be readily obtained at approximately two-week intervals if cultures are started from single non-conjugant or split-pair animals and maintained under the conditions described.

There is one case of conjugation within a clone (Gilman, 1939) in which the situation seems to have been different from that so far reported. In this clone (M, Group 2, type III) when conjugation was observed the first time in an unmixed culture, four conjugant pairs were split before they had gone through conjugation and the clones derived from the two members were tested for mating type. In all four, one member gave rise to a type III and one to a type IV clone. Since this phenomenon did not recur, it is impossible to tell whether it was due to a change of mating type as a result of autogamy or some other nuclear change or whether it resulted from an accidental contamination of the original type III culture by type IV animals from another source.

The subsequent histories of the two types isolated from this culture were very different. The four type IV clones continued to react as type IV and no conjugation occurred in them without mixture during the year they were kept under observation. The four type III clones, however, contained conjugants again approximately six weeks after the pairs were split. New split pairs were obtained from these type III clones. The clones derived from the two members of these pairs were all type III. It appears, then, that conjugation within a clone may be the result of the production of animals permanently of both types as in clone M at the time of its first spontaneous conjugation or it may occur without the production of clones permanently of two types as in the subsequent conjugation in clone M and also in clones C2, D2, D4 (type II) and P (type III).

Conditions Necessary for Conjugation

Nutrition

Some observations of considerable interest have been made on the influence of the nutritive state on the clumping or mating reaction and

on the subsequent conjugation. No detailed observations have been made with respect to this problem in Groups 3, 4 and 5. A little has been done with Group 1, but since the results obtained were essentially similar to those obtained with Group 2 and since more detailed observations were made with Group 2, only this group will be considered. In Group 2 five stages in nutritive decline associated with characteristic changes in the mating reaction have been observed. When cultures of types III and IV in these diverse conditions are mixed the following immediate behavior is observed:

(1) Animals very well fed and plump: No immediate mating reaction and no conjugants present at the end of 24 hours.

(2) Animals well fed but not markedly plump: A weak immediate mating reaction; a few animals cling together in pairs but break apart in a short time; no conjugants present at the end of 24 hours.

(3) Animals of moderate size, not well fed: Strong mating reaction; many clumps form; these later disintegrate into pairs which remain together and complete conjugation.

(4) Animals small and thin: Strong mating reaction; many clumps form; these later disintegrate, but few or none of the animals proceed to conjugate.

(5) Animals very small and starved: No immediate or later mating reaction and no conjugation.

The mixtures used in the above observations were kept for only 24 hours so that it is not known whether conjugation would have occurred in the mixtures in the first two stages if they had been kept for a longer time, but it seems probable that conjugants would form as the paramecia reached stage 3. The various stages of nutritive decline can be seen successively in a culture to which a considerable amount of food is added and the culture then allowed to decline without the addition of more food.

These observations appear significant in that they indicate that the conditions under which the mating reaction occurs are not necessarily favorable for conjugation; i.e. the mating reaction is much less sensitive to nutritive conditions than conjugation so that the paramecia give the mating reaction before they have reached the proper condition for conjugation and also after they have passed this condition.

Diurnal Periodicity

Since a diurnal periodicity in the mating reaction has been found in certain groups of *P. bursaria* (Jennings, 1939*a*) and *P. aurelia* (Sonneborn, 1938*a*), and furthermore, since it has been stated by Maupas

(1889) that *P. caudatum* conjugates at about 4:00 A.M., this question was investigated with all five groups of mating types.

In the investigation of periodicity the mixtures were examined immediately for mating reactions and twelve hours later for conjugants. In Group 1 hourly mixtures with immediate mating reactions and later conjugation were made only between 11 P.M. and 6 A.M., but since pairs just beginning to form have been observed at various hours of the morning and afternoon, it seems fair to conclude that there is no diurnal periodicity in this group. In Groups 2 and 3, mixtures followed by immediate clumping and later conjugation were made at all hours of the day and night, so that it is obvious that there is no periodicity in these two groups. In Groups 4 and 5, immediate clumping and later pairing was obtained in mixtures made between 8 A.M. and 2 A.M. Although mixtures were not tried between 2 A.M. and 8 A.M., strong mating reactions were obtained at both ends of this period and it seems probable that clumping and conjugation would have occurred if mixture had been made at these times. It therefore appears that there is no diurnal periodicity in any of the five groups of mating types so far discovered in *P. caudatum*.

Temperature

It was desired to investigate the effect of temperature upon conjugation in order to ascertain what temperature was most favorable for conjugation, whether the temperature to which the paramecia had been exposed before mixture had any effect on the number of conjugants formed, and whether any differences existed among the groups in their response to temperature. In investigating this problem all factors except the temperatures used were kept as constant as possible. One representative clone of each mating type of Group 1, Group 2, and Group 3 was used in this work. Four cultures of each mating type were left at room temperature (24° to 28° C.) for two days. At the end of this time, additional culture fluid was added and they were placed at the various temperatures—in Groups 1 and 3: 9°, 18°, 24°, and 31° C., in Group 2: 9°, 20°, 24°, and 28° C. The cultures were left at the various temperatures for forty-eight hours. For Groups 1 and 3, at the end of this time four mixtures of the two types from each temperature were placed at all the temperatures used. Thus, four mixtures from 18° were placed at 9°, four at 18°, four at 24°, and four at 31° C., and in the same manner for each of the other temperatures. In the case of Group 3 the experiment was repeated, giving a total of eight mixtures in all. In no case were any mixtures made between cultures which had previously been kept at two different temperatures; all were between two cultures kept at

the same temperature. In Group 2, six mixtures were made instead of four and the experiment was performed twice, giving twelve mixtures in all. The mixtures were examined at twelve-hour intervals and all conjugants present were removed with a pipette and the number present recorded. When no conjugants had been found in the mixtures for the three previous twelve-hour periods, the remaining animals were removed and counted. This number was used in calculating the percentage of conjugation.

The results of the experiments are given in Table II. In Group 2, it will be seen that the greatest percentage of conjugation occurred when

TABLE II

The effect of various temperatures on the number of pairs of conjugants and the percentage of conjugation in mixtures of Group 2 and Group 3 animals. The means of eight or twelve mixtures are given in the table.

Group 2

Temperature for two days before mixture	Temperature after mixture of the two types							
	28°		26°		20°		9°	
28°	1.0	2.2%	8.6	18.3%	15.0	29.4%	0	0%
26°	2.0	3.5%	8.3	18.3%	16.4	31.8%	0	0%
20°	4.2	6.7%	19.4	33.0%	33.5	54.8%	0	0%
9°	0.0	0.0%	19.7	27.5%	33.7	45.5%	0	0%

Group 3

	31°		24°		18°		9°	
31°	4.5	9.0%	73.8	86.7%	63.2	88.2%	0	0%
24°	0.0	0.0%	68.2	88.5%	54.5	83.8%	0	0%
18°	0.0	0.0%	61.2	78.5%	49.5	71.2%	0	0%
9°	0.0	0.0%	53.2	63.7%	42.2	49.7%	0	0%

animals kept at 20° C. were put after mixture at 20° C. In general, the results indicate that the temperature at which the animals were kept both before and after mixture affect the amount of conjugation. Either very high or very low temperatures after mixture either decrease the percentage of conjugation markedly or prevent it entirely. Low temperatures (9° or 20°) before mixture appear to be more favorable for conjugation than high temperatures (26° or 28°).

In Group 3, the highest percentage of conjugation occurred when mixtures of animals from 24° C. were kept at 24° C. In general, it can be said that, as in Group 2, very high or low temperatures after mixture are unfavorable for conjugation. Unlike Group 2, high temperatures before mixture appear to be more favorable than low.

When the numbers of conjugants formed in each of the mixtures

during each of the twelve-hour periods after mixture until no more conjugants were formed were considered, it was found that in Group 2 most of the conjugants were formed in the first twelve hours after mixture. In only one set of mixtures,—those put at 20° after two days unmixed at 9°,—is the time of greatest conjugation shifted to the period between twelve and twenty-four hours after mixture. At 24° in Group 3 most of the conjugants were found between twelve and twenty-four hours after mixture while at 18° most of the conjugants were found 36 to 48 hours after mixture.

In Group 1, the results obtained are not complete enough to justify inclusion but they indicate that the lower the temperature before mixture the greater the conjugation, and that most conjugants are produced when the paramecia previously kept at 9° C. are put at 20° C.

Group Differences

An attempt was made to ascertain whether there were any differences between the groups besides the primary group difference in the mating type. With respect to size, it was found that the animals in Group 3 are characteristically smaller than the animals in Groups 1, 2, 4 and 5. Under isolation line conditions, these differences are hardly noticeable but when grown in mass cultures become very striking. In Group 1, it was found that on the average the type II animals were smaller than the type I and of a slightly different shape, being rather shorter and broader. This difference becomes very obvious in conjugating pairs where the type I members may frequently be twice as long as the type II member. So far only two collections of eight clones in all have been found for Group 1 so that it is possible that the condition is not general for the group. However, all the clones so far examined (three of type II and five of type I) show the difference. In Group 2 characteristic size differences between clones have been observed but without any correlation with mating type.

There are also characteristic differences in Group 1 as to selfing. Type II animals self readily while the type I animals self very rarely. In Group 2, both selfing and non-selfing clones are found but they are not correlated with mating type. Group 3 seems to have rather less selfing than either of the other two groups since only a few instances of conjugation without mixture have been found in this group.

Under good conditions for conjugation, there are, as mentioned previously, differences in the proportions of the animals in mixtures which conjugate. Thus the greatest proportion of conjugation in mixtures occurred in Group 3 and the lowest proportion of conjugation in

Group 1. Another already-mentioned difference with regard to conjugation is that low temperature before mixture causes more conjugation in Groups 1 and 2 but less conjugation in Group 3.

A comparative study was made of the adverse effects on *P. caudatum* of mixture with three clones of *P. aurelia* (H, G, and 47). These clones are known (Sonneborn, 1938*b*, 1939, and unpublished) to cause certain other clones of *P. aurelia* to die in characteristic fashion when they are mixed with them. These three clones were mixed with 73 clones of *P. caudatum* and the mixtures were observed daily until dead or until all the other mixtures which showed an effect were dead. In all cases, control groups of *P. caudatum* were kept without mixture with the clones of *P. aurelia*.

The effect on *P. caudatum* which is produced by G is first indicated by the avoiding reaction and spinning by the affected individuals. The spinning takes the form of rapid rotation on the longitudinal axis with little or no forward movement. In some instances, the animals revolve (without moving forward) around an axis parallel to the longitudinal axis of the paramecium so as to describe a cylinder or a segment of a cone. These manifestations do not occur continuously but alternate with periods of quiescence or normal swimming. In addition to the alteration in behavior described above, morphological changes also occur; the paramecia become thin, flattened and gradually become transparent. As they become transparent, crystals become visible in the cytoplasm. There is no regularity about the position of the crystals, which are sometimes in the anterior end, sometimes in the posterior end, and sometimes distributed about the periphery. No characteristic differences in the reaction to G were noted between the groups of mating types.

Stock H has no marked effect on behavior of *P. caudatum* but the morphological changes are much more striking than those produced by Stock G. The affected animals stop feeding and lose all their food vacuoles. They then become filled with clear vacuoles. Generally there were two large vacuoles in each animal in the region of the contractile vacuoles and several smaller vacuoles. In some, only one large vacuole was formed. The vacuoles gradually enlarge until the ectoplasm becomes widely separated from the rest of the cytoplasm and the paramecia appear blistered. Frequently, just before death, the macronucleus becomes visible as a round body. In many dead animals both the blistering and the visible macronucleus are evident while in others only one is to be seen. In other cases, only the vacuolization was apparent. No characteristic differences were noted among the first three groups in their response. However, both Groups 4 and 5 were resistant to the effect of H and remained normal.

The effect of Stock 47 was as striking as that of H in a somewhat different way. The first effect was that the animals became shorter and thicker than normal. This was followed by enlargement of the posterior end. This enlargement appeared to be caused by the massing of the cytoplasm and macronucleus at the posterior end toward one side of the animal. The animals frequently became almost spherical before death. In some instances a single vacuole appeared at the posterior end but this was not a constant effect. Much more common were small vacuoles under the ectoplasm giving the animals a rough appearance. None of the clones tested were resistant to Stock 47, and it is of considerable interest that the clones which were resistant to H appeared to be more quickly and strikingly affected than the other clones.

In summary, it can be said that there appear to be characteristic differences between the groups in size and in various physiological characteristics. In Group 1, there also appear to be characteristic differences between the two mating types in size. In the other groups, there were no such characteristic differences between the mating types.

Discussion

The condition in *P. caudatum* with respect to the number of types within a group is like that in *P. aurelia,* since only two mating types have been found in each group; not four or eight as in *P. bursaria. P. caudatum* differs from both *P. aurelia* and *P. bursaria* in that it has certainly four and in all probability five groups of mating types while they have only three groups.

The wide occurrence, under certain conditions, of conjugation within a clone is of considerable interest in connection with the question of whether or not the presence of both mating types is necessary for conjugation to occur. In one clone (M) when pairs were split, it was found that both mating types of the group were indeed present. This may have been due to a change of mating type at autogamy, such as was found to occur in *P. aurelia* by Sonneborn (1937) and Kimball (1937). The possibility of accidental contamination cannot be excluded, however. In all the other clones of *P. caudatum* in which it was possible to separate conjugating pairs, the cultures derived from both members of a split pair were of the same type as the original culture. It has been impossible as yet to ascertain whether two mating types were present at the time conjugation occurred, one of which changed type again to become the same as the original clone or whether conjugation was occurring between two animals of the same mating type. Kimball (1939) has shown that in *P. aurelia* there may be a temporary change of mating type

during vegetative reproduction but that animals which have thus changed their mating type give rise to clones of the original mating type. It appears possible that a similar process occurs in *P. caudatum*. That the repeated occurrence of conjugation within a clone was the result of a preceding autogamy appears unlikely in view of the fact that the two members of split pairs gave rise to clones of the same mating type and in view of the failure to find evidence of nuclear change in these clones. In some clones selfing occurred frequently while in others it occurred only once or not at all. This difference between clones is probably the basis of the conflicting results on the effect of environmental changes on conjugation in *P. caudatum*. Zweibaum (1912) found that he could induce conjugation in the clone of *P. caudatum* with which he was working by adding various salts in certain concentrations. He concluded, since he used only this one clone, that conjugation was dependent solely on environmental factors. Hopkins (1921) and Ball (1925) tried Zweibaum's methods on a number of clones of *P. caudatum* and found that they gave conjugation with some clones but not with others. These conflicting results can be explained by assuming that Zweibaum had a clone of animals in which selfing occurred rather readily, while Hopkins and Ball, since they used several clones, had some which selfed readily and some which would not self.

The nutritive requirements observed for conjugation appear to agree very well with those reported by Maupas (1889), Calkins and Cull (1907), Jennings (1910), Zweibaum (1912), Calkins and Gregory (1913), Ball (1925), Chatton and Chatton (1931), and Giese (1935). The paramecia conjugate when not too well fed, yet not starved. This is, of course, the condition produced when the food supply is suddenly decreased or when animals which have exhausted the food in the medium are given a limited supply of food. The most interesting result of the work on the effect of nutrition on conjugation is the fact that the mating reaction, clinging together and clumping, occur under conditions which are not satisfactory for conjugation. This phenomenon of strong clumping with the production of few or no pairs of conjugants was observed in several mixtures of clones in Groups 1 and 2. It is especially marked in those animals which have passed the point of optimum nutritional condition for conjugation and somewhat less so when the animals have not yet reached this condition.

Summary

1. An investigation of the numbers and interrelations of the mating types in *Paramecium caudatum* in cultures derived from single animals

isolated from wild cultures was carried out. Of 93 clones from 26 natural sources the mating types are still to be identified in 3 clones from 2 natural sources.

2. The clone cultures could be divided into mating types in several non-interbreeding groups. Animals from cultures of different groups did not conjugate when mixed with one another. Within each group two mating types were found. Animals from cultures of different mating types belonging to the same group conjugated when mixed together. Four non-interbreeding groups of mating types have been definitely established and the occurrence of five groups is highly probable.

3. In regard to the geographical distribution of the mating types, no evidence was found for the formation of local groups of mating types which would not conjugate with animals from other localities.

4. Ordinarily, conjugation occurred only when animals from two different mating types were mixed but under certain conditions some clones conjugated without mixture.

5. It was found that ordinarily such conjugation was not the result of the production of two mating types at autogamy as in *P. aurelia.*

6. In one case (clone M, type III) both mating types of a group were produced in a clone but it was not possible to correlate this fact with a preceding autogamy.

7. It was found that the mating reaction itself (clumping of the animals) would occur under nutritive conditions which would not permit the completion of conjugation.

8. The temperature both before and after mixing the mating types has a definite effect on the proportions of the animals conjugating. Very little or no conjugation occurred when the animals were kept at the extremes of temperature (9° and 28° and 31°) after mixture. A low temperature prior to mixture caused more conjugation in Group 2, less in Group 3.

9. None of the five groups of mating types gave any indications of a diurnal periodicity.

10. Group 3 animals are obviously smaller than Group 1 or Group 2 animals.

11. In Group 1, there is a difference in size between the mating types; type I is larger than type II.

12. Type II animals "self" (conjugate without mixture) much more frequently than type I animals.

13. In mixtures between animals of different types the largest percentage conjugate in Group 3 and the smallest in Group 1.

14. No differences were found between Group 1, 2, and 3 in their response to the toxic effects produced by races G, H, and 47 of *P.*

aurelia. The clones which form Groups 4 and 5 are resistant to the lethal effect of H.

15. A possible explanation of the conflicting results on the effect of environmental factors on conjugation obtained by earlier workers on *P. caudatum* was presented.

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